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# MASS REARING OF THE CEREAL LEAF BEETLE IN THE LABORATORY[1/]

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Expanded research with the cereal leaf beetle, Oulema melanopus (L.), caused us to develop methods of rearing large numbers of all life stages for laboratory use. Castro, by using covered lamp chimneys over 4-inch pots of oats for adult feeding and oviposition and uncaged 6-inch pots of oats for larval feeding, was able to rear limited numbers of beetles.[3/] However, numerous beetles were injured by the handling necessary to transfer the beetles from pot to pot, and the general results were inconsistent. Connin and others, therefore, made a study of rearing methods in 1965 in which they used adult beetles collected in the field in Berrien County, Mich.[4/] Further studies by Schillinger and Connin then resulted in a rearing program that has met the demands of researchers and has produced an excess of several thousand beetles a month.[5/]

## FACILITIES

The rearing facility consists of a 60-by 12-foot basement room in a headhouse-greenhouse complex. Three shelves extend along both long sides of

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1/ This was a cooperative study with the Entomology Department, Michigan State University.

2/ R. V. Connin, D. L. Cobb, and G. Lawson are with the Entomology Research Division, Agricultural Research Service; J. C. Arnsman is with the Entomology Department, Michigan State University.

3/ Castro, T. R. Natural History of the Cereal Leaf Beetle, Oulema melanopa (Linnaeus), and Its Behavior Under Controlled Environmental Conditions. (Unpublished doctor's thesis, Michigan State University.)

4/ Connin, R. V., Cobb, D. L, Arnsman, J. C., and Gomulinski, M. S. Laboratory Rearing of the Cereal Leaf Beetle, Oulema melanopus (L.). U. S. Dept. Agr., Agr. Res. Serv., Ent. Res. Div. Spec. Rpt. W-22.

5/ Schillinger, J. A., and Connin, R. V. Adult Preference, Ovipositional Behavior, and Larval Growth as Laboratory Screening Techniques for Resistance of Small Grains to the Cereal Leaf Beetle. Jour. Econ. Ent. (in press).

the room. Mounted above each shelf is a series of 48-inch reflector-type fluorescent fixtures having two 40-watt cool white tubes (figure 1). Time-clocks maintain a 16-hour photoperiod, and light intensity at plant height is 300 foot-candles.

Figure 1.--General View of rearing room showing arrangement of shelves and light.

The room has humidifiers at each end, fin-tube hotwater radiators, fans for air circulation, and three window-type air conditioners. With the thermostat set at 76°F., the heat is always on and is always overcome by the constantly operating air conditioners. Thus temperatures remain constant at 78° during the 16-hour light period and at 74° during the 8-hour dark period. Relative humidity remains constant at 60 percent.

General laboratories, growth cabinet rooms, storage, and greenhouse space are adjacent to the rearing room.

## REARING PROCEDURES

### Oviposition Cages

The cages used to confine ovipositing adults are 16 by 16 by 16 inches and are constructed of 3/4-inch plyboard on the bottom, front, and back and of 1/8-inch plexiglass on the remaining two sides and top. The wooden front has a hinged door, and wood stripping is tacked to the inside of the opening to provide a tight seal. The plexiglass sides and top are fastened to the bottom, back, and front with wood screws; where plexiglass edges meet, they are glued. Two-inch holes covered with nylon screen placed in the plexiglass sides and top provide ventilation. The cage is designed to provide maximum illumination and a minimum of hiding places for the beetle (figure 2).

Figure 2.--Oviposition cage.

Four 4-inch pots of seedling plants (oats or barley) about 5 inches tall are placed in the oviposition chambers with about 200-unsexed, post-diapause adults. The normal sex ratio is about 1:1. If the eggs are to be used in the rearing program, the pots are changed daily. If the eggs are desired for more specialized studies, the pots may be changed at any interval to provide eggs of suitable ages, or lantern globe cages may be placed over individual pots. We use a globe that has a height of 7 inches and a base diameter of 3 3/8 inches so that the bottom of the globe can fit snugly into the 3 1/2 inch plastic pot. The top is covered with nylon cloth, and a layer of plaster of paris is placed over the soil so that the cage and pot can be tipped to shake the adult beetles into the inverted globe.6/ The pot of plants with its eggs can then be removed, another can be inserted, and the pot and the cage can be set right side up without any loss of insects.

Oviposition usually occurs in these cages within 7 to 10 days after post-diapause adults are removed from storage, and it continues 6 to 8 weeks. Females lay from 150 to 200 eggs each; average viability is about 80 percent.

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6/ Connin, R. V., Cobb, D. L., Arnsman, J. C., and Gomulinski, M. S. Plaster of Paris as an Aid in Rearing Insects Pupating in the Soil. Jour. Econ. Ent. (59)6: 1530. 1966.

## Larval Development

The pots of plants containing eggs are removed from the oviposition chambers and placed on large metal trays. Dividers in the trays make it possible to separate the material by age groups. Water is placed in the trays to maintain a high relative humidity and to eliminate watering large numbers of pots by hand (figure 3). The eggs hatch in 4 to 7 days, and the larvae are allowed to feed on the plants until they reach the second instar, normally within 10 days.

Figure 3. --View of metal trays used to hold pots of plants containing incubating eggs and first-instar larvae.

When most of the larvae on any group of plants have reached the second instar, the plants are cut at the base and foliage containing about 250 larvae is spread over oat or barley seedlings 5 to 6 inches tall growing in 16-by 22-by 3-inch metal greenhouse flats (figure 4). Just before the infestation (figure 5), the soil in these flats is covered with a thin layer of plaster of paris.7/ When it dries, a layer of white quartz sand 1/2 to 3/4 inches deep is poured on top. The layer of plaster prevents the prepupae from entering the soil and forces them to pupate in the sand where they are easily recovered.

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7/ Connin, R. V., Cobb, D. L., Arnsman, J. C., and Gomulinski, M. S. Plaster of Paris as an Aid in Rearing Insects Pupating in the Soil. Jour. Econ. Ent. (59)6: 1530. 1966.

Figure 4.--Infesting flats of seedling barley with cereal leaf beetle larvae.

Figure 5.--Plastering soil of a flat of barley.

Second-instar larvae placed on flats of seedling grain complete their development to the pupal stage in 10 to 14 days without further transfer to fresh plants. Therefore, to facilitate handling large amounts of material, we allow an arbitrary time of 12 days after infestation for feeding though a few prepupae may still be found at this time.

Figure 6.--Washing sand from a flat to recover pupae (note layer of plaster over soil).

At the end of the larval feeding period, the foliage is cut as close as close to the sand layer as possible, and the sand is washed from the flat through a 16-mesh sieve that catches the loose pupal cells (figure 6). Most of these cells are loosely attached to the plaster layer and can be easily removed with a vacuum aspirator set at about 5 inches of vacuum (figure 7).

One flat with moderately heavy seeding that is infested when the plants are about 6 inches tall can support more than 300 larvae to pupation. However, crowding is avoided by placing only an average of 200 to 250 larvae on each flat. Generally this number is estimated, but counts are made periodically for accurate determination of the percentage pupation. Average pupation for all infested flats is about 81 percent.

Little mortality of larval forms has occurred. The critical period appears to be the time of pupation. Thus the amount of moisture in the sand layer should be limited before pupation.

## Handling of Pupae

Pupae from the larval-feeding trays are collected in groups of several hundred and placed on a plaster layer covering the soil of 4-inch pots of seedling barley and oats. The pots are held in 32-mesh-plastic-screen-wire

Figure 7.--Collecting pupae with vacuum aspirator.

cages similar in design to the oviposition cages. Adults emerge from the pupal cells in 6 to 8 days; average emergence is 63 percent.

Adults emerging from the pupal cells feed on the potted plants for 24 to 26 days; additional pots are added as needed. If the beetles begin to seek hiding places, paper towels folded in accordion pleats are placed in the cages. Survival of these prediapause adults averages 89 percent; as noted, most mortality occurs shortly after emergence from the pupal cell.

When feeding has almost stopped (28 to 30 days after emergence from the pupae), all the living beetles are collected from the cages and placed in pint plastic freezer containers (250 to 300 beetles per container) (figure 8); crumpled paper toweling is moistened in a 5 percent solution of sodium-hypochlorite and placed in the containers to inhibit formation of mold. The containers are stored in a refrigerator at 38°F. for 3 to 4 months after which the beetles are used to continue the rearing cycle and for other research activities. Refrigerated beetles suffer little mortality and have been stored as long as 13 months with a 95-percent survival.

Both cardboard ice cream containers and freezer containers have been used. However, the cardboard containers dry out if they are not remoistened periodically, and the beetles can desiccate. No additional care is required when plastic containers are used.

## Diapause

The cereal leaf beetle overwinters as an adult and seeks shelter in such places as field trash, corn stubble, cracks and crevices of buildings,

Figure 8.--Beetles stored in plastic dishes in refrigerator.

and posts. Overwintered adults appear in the spring; they mate; and the females lay eggs on the upper surfaces of host plant leaves. In Michigan, overwintered adults become active in late March. Eggs are produced in late April and early May, and summer adults are found in mid-June.

In the laboratory, a few adults mate and produce a few eggs before cold storage, but usually only when they are under stress. However, the few eggs that are laid are usually not viable. Hoopingarner and others indicated that a diapause of some sort is required for egg production.8/

The constant demand for large numbers of beetles has caused us to "follow the nature of the beetle" in the rearing program. Consequently, most adults are placed in cold storage to complete diapause. However, recent attempts to break diapause by hormonal treatment indicate that it may soon be possible to eliminate cold storage.9/

Today, 6 weeks storage at 38°F. is required before any mating and oviposition will occur. Our host plant resistance studies, in which paired

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8/ Hoopingarner, R. A., Kumararaj, S., and French, A. L. Gametogenesis and Radiation Effects in the Cereal Leaf Beetle, Oulema melanopa. Ann. Ent. Soc. Amer. 58(6): 777-781. 1965.

9/ Connin, R. V., Jantz, O. K., and Bowers, W. S. Termination of Diapause in the Cereal Leaf Beetle by Hormones. Jour. Econ. Ent. 60(6): 1752-1753, 1967.

entries were tested with similar numbers of female and male beetles, indicated that a minimum of 12 weeks storage was required for consistent egg production.[10/] Other tests we made in which adults of the same age were taken from cold storage every 2 weeks indicated that oviposition was quicker and greater as the time in storage was increased up to 5 months. Therefore, a minimum 3-month period of cold storage has been established for the rearing program to insure consistent behavior in postdiapause adults used in various research projects.

## SUMMARY

Procedures have been developed for producing eggs, larvae, pupae, and adults of the cereal leaf beetle in the laboratory.

Postdiapause adults caged in 16-by 16-by 16-inch plastic cages oviposit on potted seedling oats or barley. After the larvae feed on the potted grain for several days, they complete their development on flats of seedling grain that contains soil covered with plaster of paris and sand. Recovered pupae are held in screen cages for adult emergence. Prediapause adults are stored in plastic refrigerator dishes for 3 to 4 months at 38°F. and are then available for further use.

The rearing facilities at Michigan State University are producing more than 10,000 beetles per month. Demands of research projects for all life stages can be met. The stock culture can be increased by more than 3,000 adults per month, which may eliminate dependence on field collections in the summer.

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[10/] Schillinger, J. A., and Connin, R. V. Adult Preference, Ovipositional Behavior, and Larval Growth as Laboratory Screening Techniques for Resistance of Small Grains to the Cereal Leaf Beetle. Jour. Econ. Ent. (in press).

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